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OBSERVATIONS UPON A MASTITIS BACILLUS.

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In the *Agricultural Year-Book of Switzerland* for 1888, Professor Hess, of Berne, published the results of an inquiry into the causes of mastitis in cows. He proved that the infectious forms of mastitis are due to several and distinct kinds of bacteria, which penetrate through the milk canals into the milk glands. Within the latter they find a soil suitable for their growth, and for the development of their specific pathogenic properties. The resulting inflammation of the tissues is at times so mild, and the alterations in the secretion so slight, that the affected cows can still be milked, and the milk used for domestic purposes or for the manufacture of cheese. The milk from one affected cow, by being mixed with milk from healthy cows, can infect large quantities with the active bacteria. The determination of the nature and the action of these bacteria is, therefore, of hygienic and economic importance.

On the suggestion of Professor von Nencki, I undertook the investigation of the chemical products of one of these specific bacteria. In the first place, its causal relation to the disease had to be established. This I was able to do through the kindness and with the assistance of Professor Guillebeau, Berne. A cow suffering from mastitis was slaughtered, and cultures made from the contents of the udder immediately after death. A micro-organism was isolated in pure culture and handed over to me for investigation. It was a short rod bacillus with rounded ends, though occasionally forms were present closely resembling micrococci. Its average length was 1μ. In gelatine stabcultures it formed a dull white growth along the line of inoculation. On the surface the growth was abundant, greyish white, and with an irregular margin. The surface growth adhered firmly to the gelatine, and was viscous. Portions removed with a platinum needle could be drawn out into long threads. In old cultures the superficial growth was meshed and netlike. The bacillus did not liquefy gelatine. It grew

well on the usual culture media, and was easily recognisable by its microscopic appearance and its characteristic growth in gelatine.

Its specific pathogenic properties were proved by the following experiment made on a healthy goat. The goat is well adapted for such experiments, as fluids can be injected into its udder with ease and certainty:—

Five drops of a pure broth culture of the bacillus were injected into each teat. On the day after the injection the animal ate less than usual, and had shivering attacks. The udder was enlarged, firmer, and hot. Milking gave pain, and the milk was ropey and viscous—after twenty-four hours, curdy. The animal, in walking, stretched the hind legs wide apart, as contact with the inflamed udder was painful. In one of the milk-glands there was a considerable quantity of gas. After the third day the symptoms began to diminish, and on the eighth day the inflammation had subsided. Samples of the milk were taken, and gelatine plates inoculated from them. They yielded pure cultures of the bacillus.

It is important to notice that when a natural infection takes place in the stall, the first sign of the inflammatory process is given by the milk itself. The milk contains small coagula before the symptoms of udder inflammation appear. After the inflammation has subsided it is some time before the milk acquires its normal properties. During the inflammation the milk has an acid reaction, is curdy, and contains an increased amount of inorganic matter. The amount of milk-sugar is greatly lessened. The amount of fat is also lessened. The bacteria can also be found in the milk during this period.

Having proved the pathogenic nature of the bacillus, and its causal relation to the mastitis, its action upon carbohydrates, proteids, and fats was tested.

1. *Carbohydrates.*

To 2 litres of beef-broth were added 100 grammes grape-sugar and 50 grammes carbonate of lime. The fluid was sterilised in an autoclave at 120° C., and then inoculated from a pure culture of the bacillus. The air in the flask was replaced

by carbonic acid gas, and the flask placed in an incubator at 38° C. After twenty-four hours there was an active development of gas, and the fluid was frothy. On the third day the first gas samples were collected in an eudiometer tube over mercury. The result of the gas analyses was as follows:—

2 Litres 5 per cent. Grape-sugar Solution in CO_2.

	3rd Day. Active Development of Gas.	5th Day. Weaker Development of Gas.	14th Day. Fermentation nearly ended (about 100 cc. Gas in 24 hours).
Reduced Volume.			
Amount of Gas, .	cc. 51·359	cc. 37·04	cc. 49·36
After adding Potash,	11·99	5·94	0·37
After adding Oxygen,	59·263	20·94	5·42
After Explosion, .	41·00	12·15	4·90
After adding once more Potash, .	41·01	12·15	4·89
Result in percentage Volume.			
CO_2, . . .	76·65	83·96	99·25
H, . . .	23·68	16·00	0·72

The gases were, therefore, CO_2 and hydrogen, the latter in decreasing amount. At the beginning of the fermentation more than one-third of the gas was hydrogen; after five days, one-fifth; and after fourteen days, only 0·72 of the percentage

volume, whilst the amount of CO_2 was 99·25 per cent. Methan gas was not present.

Ærobic cultures were also made in sugar broth. In both cases the fluid was examined on the 20th day, and the purity of the cultures tested. In each case some unchanged sugar was present. The fluid was tested with Fehling's solution and then examined in Wild's polaristrobometer. Whilst, however, the amount of unchanged sugar in the ærobic cultures was at most 0·5 grm., the amount in the anærobic was 38·1 grm. In the ærobic flasks there was always an almost complete fermentation of the sugar. The bacillus was facultative anærobic, and like the yeast was most active with a limited supply of atmospheric oxygen.

A portion of the fluid, tested with iodine and caustic soda, gave a distinct precipitate of iodoform. The entire fluid was therefore distilled till no further reaction was given with iodine and soda. The distillate was again distilled down to half its volume, and the process repeated after adding sodic chloride. The final distillate was saturated with carbonate of potash. A thin yellow layer of alcohol collected on the surface. The amount was small and was just sufficient for fractional distillation. It distilled between 74° and 80° C. The odour and the test with benzoylchloride and potash proved it to be ethyl-alcohol. Higher boiling alcohols were not present.

Oxalic acid was added to the remainder in the retort, and then distilled. The distillate contained the volatile fatty acids. It was saturated with ammonia, and concentrated on the water-bath till all smell of ammonia had disappeared. A portion treated with alcohol and sulphuric acid developed the characteristic odour of acetic ether. The entire fluid was precipitated with nitrate of silver, quickly filtered, and the precipitate dried in a dessicator in the dark. The salt was acetate of silver, according to the following analysis:—0·079 grm. of the silver salt gave on combustion 0·051 grm. = 64·55 per cent. silver. The volatile acid was therefore acetic acid.

The remainder in the retort was concentrated and extracted with ether. After distilling off the ether a yellow syrupy fluid remained, which was diluted with water and boiled with

zinc-hydroxide. On analysis it proved to be lactic acid. 0·63 grm. of the salt dried at 110° C., lost 0·0476 grm. in weight = 18·1 per cent., and 0·2154 grm. of the dried salt gave on combustion 0·072 grm. ZnO = 26·84 per cent. Zn.

It was, therefore, the inactive ethylidene-lactic acid $(C_3H_5O_3)_2Zn + 3\ H_2O$—which, as zinc salt, contains 18, 18 per cent. water of crystallisation and 26·75 per cent. Zn.

The ærobic and anærobic cultures gave the same decomposition products.

I may mention here that the bacillus also decomposes glycerine, with and without atmospheric oxygen. The fermentation was accompanied by an active development of gas which lasted fourteen days.

The decomposition products of sugar are—(1) Inactive lactic acid as chief product; (2) acetic acid; (3) ethylalcohol; (4) gases, CO_2 and hydrogen—the latter in constantly decreasing amount.

2. *Fat.*

To 1 litre neutral meat broth was added 2 per cent. of fat, then sterilised and inoculated with the mastitis bacillus. After a month the fluid was tested by Hofman's method. The results were negative—the fat remained undecomposed.

3. *Proteids.*

Two litres of water were added to 500 grm. finely minced meat—the whole sterilised and inoculated with the bacillus. The air was replaced by CO_2. An eudiometer tube was attached to collect the gases. No gas developed, and there was no evident decomposition of the albumen. The ærobic cultures also gave negative results.

Filtered bouillon cultures of the bacillus proved harmless to animals, and the bacillus subcutaneously injected, produced no pathogenic effect. The bacillus, therefore, belongs to the large class of bacteria which requires a soil containing carbohydrates for the full development of their ferment activity. In the milk the sugar is split up by it, and alcohol, acetic and lactic acids result. The acids, and especially acetic acid, by irritating the glandular tissue, probably give rise to the strictly localised inflammation of the udder. The first condition for the inflam-

mation is a chemical change in the milk itself—which is brought about by the bacteria—with the formation of the above-mentioned products.

This bacillus is also a cause of *boursouflement des fromages*, an abnormal fermentation which lessens greatly the market value of Swiss cheese. I refer here to the cheeses made in the Emmenthal near Berne.

The following experiment was made in Dr Freudenreich's laboratory. Control cheeses, made from 10 litres of milk, were inoculated from a pure culture of the bacillus, at the moment when the rennet was added, and kept at the usual temperature, viz. 16° C. A control uninoculated cheese was made at the same time. (The cheese contains about 2·5 per cent. milk sugar.)

The inoculated cheeses became full of large holes and diminished in weight; the uninoculated cheese presented a normal appearance.

At times these abnormal fermentations give rise to an inflammable gas, which, according to my gas analyses is Hydrogen.

I finally tested the effect of the abnormal milk upon animals.

Flasks containing 1 to 2 litres milk were sterilised, inoculated with the mastitis bacillus and kept at 38° C.; uninoculated milk served as control. The milk was inoculated in the evening. Next morning there was an active development of gas, and the milk was frothy. In eighteen to twenty hours the milk contained coagula, and in twenty-two to twenty-six hours the coagulation was complete. The control milk was unchanged. The reaction of the milk was always markedly acid. At the ordinary room temperature, the coagulation was slower, and only complete after four to five days. The optimum temperature was 37° to 38° C. This is probably due to the fact observed by Warington (*Lancet*, 1888, vol. i.), that the quantity of acid necessary to coagulate milk is smaller the higher the temperature.

An adult dog—15 kilogrm.—was fed with the fermented milk. The result was negative. The milk was then mixed with soda to neutralise the acid of the gastric juice, but again with negative results. On the other hand, kittens fed with the milk had violent attacks of diarrhœa. After one to two days they recovered. Thus, whilst the milk had no injurious effect

on the adult dog, for the kittens it was always injurious and produced diarrhœa.

Milk from cows infected with this bacillus might therefore easily cause diarrhœa in children.

The bacillus grows well in gelatine containing 2 per cent. bile, and also in sterilised ox bile, a factor which would favour its growth and development in the intestine.

The above researches prove that this bacillus can produce:—

1. An inflammation in the udder of cows.
2. An abnormal milk secretion.
3. An abnormal fermentation of cheese.
4. Diarrhœa in young animals, and may therefore be a possible cause of diarrhœa in children.

The bacillus retains its vitality for a long time—after six months the cultures still retained their ferment activity.

The bacillus is probably only one of a large class of bacteria capable of producing mastitis. It would be interesting to investigate in what way other bacteria capable of producing this disease act. Is the inflammation due more to their fermentative activity than to any distinct pathogenic properties of the cells themselves? Bacteriological chemical investigations would not only decide this—they would at the same time aid us in distinguishing physiologically micro-organisms which often closely resemble one another morphologically.[1]

[1] It is a pleasant duty to acknowledge here my great indebtedness to Professor von Nencki for the invaluable advice and help so ungrudgingly given in this and other work carried out in his laboratory.